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Acrylonitrile exposure in the general population following a major train accident in Belgium: A human biomonitoring study

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HIGHLIGHTS

- *N*-2-cyanoethylvaline (CEV) was measured after a train accident with acrylonitrile.
- 37% of the evacuated non-smoking residents exceeded the CEV reference value.
- Some extreme CEV concentrations were observed, e.g. more than 1000 pmol/g globin.
- The highest exposures were observed in humans living along the sewage system.

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ABSTRACT

Background: On Saturday May 4, 2013, a train transporting chemicals derailed in the village of Wetteren (Belgium) and caused a leak of acrylonitrile (ACN).

Objectives: To assess the human exposure to acrylonitrile in the local population with the highest suspected exposure.

Methods: Between May 18–25, 242 residents participated in the study. *N*-2-cyanoethylvaline (CEV), a biomarker that is highly specific for ACN exposure, was measured in the blood. To account for potential influence by smoking, cotinine was determined in the urine. Participants also filled in a short questionnaire.

Results: In the evacuated zone, 37.3% of the non-smokers and 40.0% of the smokers had CEV concentrations above the reference values of 10 and 200 pmol/g globin, respectively, at the time of the train accident. Spatial mapping of the CEV concentrations depending on the residential address showed a distribution pattern following the sewage system.

Discussion and conclusion: The train derailment resulted in a highly atypical sequence-of-events. In addition to exposure in the direct vicinity of the site of the train derailment, exposure also occurred via the sewage system, into which acrylonitrile had entered shortly after the accident.

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1. Introduction

On Saturday, May 4, 2013, around 2 AM, a freight train transporting chemicals derailed in the village of Wetteren (East-Flanders, Belgium). Several rail tank cars containing in total 60 t of acrylonitrile (ACN) exploded and immediately a fire developed. In addition to the formation of toxic vapours of ACN, other toxic gases such as hydrogen cyanide and nitrogen oxides were released due to the fire-induced decomposition of ACN. The water used to extinguish the fire drained into the sewers, resulting in a further distribution of ACN and by-products of the combustion via the sewers. One resident died, one resident experienced cardiac arrest but was successfully resuscitated, one resident developed deep coma, around two hundred residents were hospitalized, and more than 2000 residents were evacuated. The provincial phase of the disaster plan was proclaimed. The evacuation period varied from three days for the first residents that were allowed to go home until almost three weeks for the residents living close to the accident site.

ACN (C_3H_3N) is a volatile, flammable, water-soluble, colourless liquid used as an intermediate in the manufacturing of acrylic fibers, styrene plastics and adhesives. It has a garlic or onion-like odour (European Commission, 2004) and its vapours are heavier than air and may thus travel along the ground over a long distance. The toxicodynamics of ACN have been extensively reviewed elsewhere (ATSDR, 1990; European Commission, 2004; DFG, 2007). Signs of acute toxicity include respiratory tract irritation and central nervous system dysfunction, resembling cyanide poisoning, which may lead to loss of consciousness or even death. With respect to chronic toxicity, there is sufficient evidence in experimental animals for the carcinogenicity of ACN. IARC (1999) considered that there is inadequate evidence in humans for the carcinogenicity of ACN and therefore the substance has been categorized as possibly carcinogenic to humans (Group 2B).

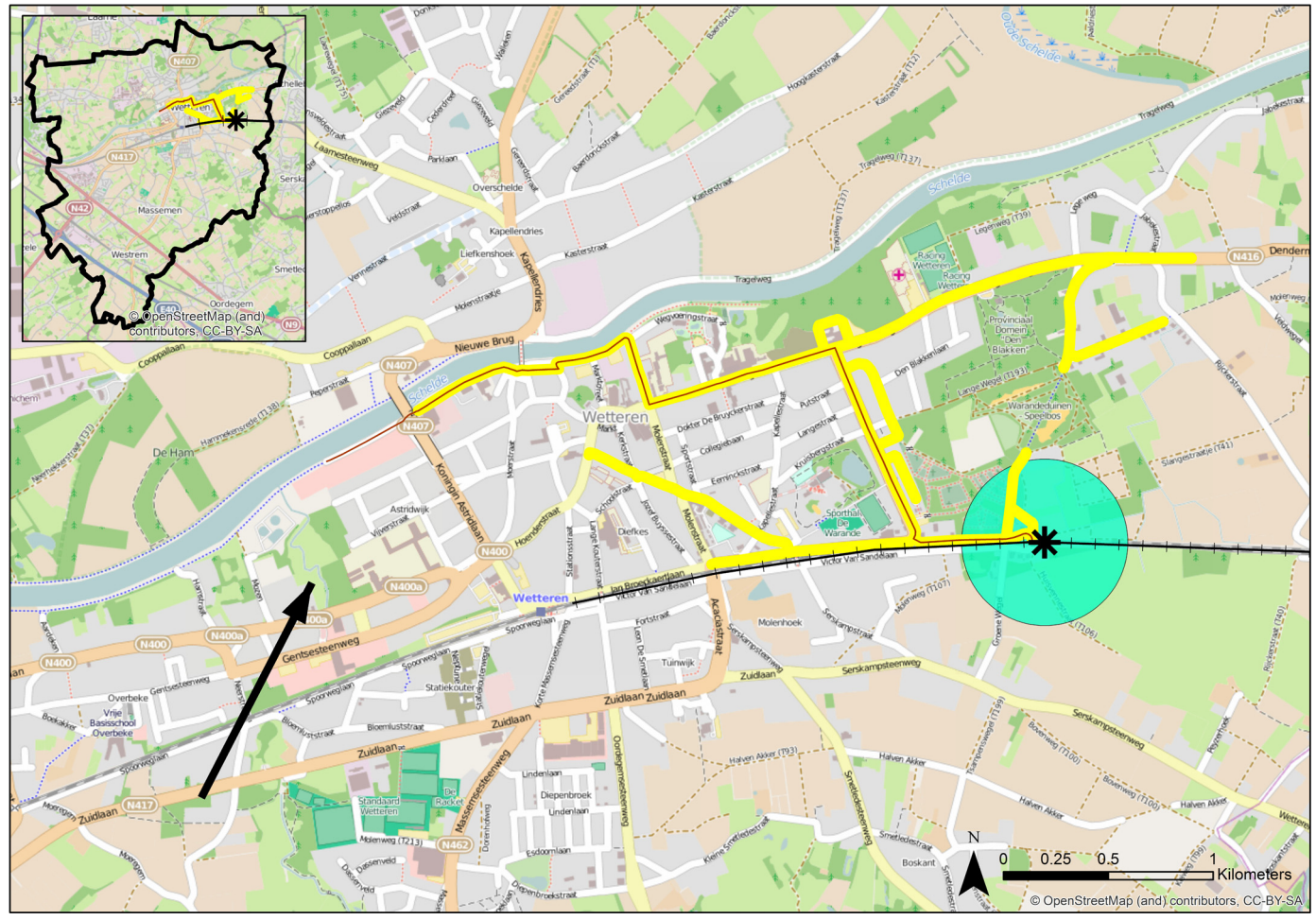

Fig. 1. Study area for the biomonitoring study in the local population.

Train accident.

Railroad.

Sewage system.

Prevailing wind directions at the moment of and in the days following the accident.

Zone 1 (EZ1): 250 m perimeter of the evacuation zone that was evacuated at night in the hours following the accident.

Zone 2 (EZ2): streets parallel with the sewage system and downwind of the train accident that were evacuated later, i.e. in the days following the accident.

Zone of 'Controls': the commune of Wetteren, EZ1 and EZ2 excluded.

Due to their electrophilicity, ACN and its epoxide react with nucleophilic sites in DNA or other macromolecules to form adducts (SCOEL, 2003). *N*-2-cyanoethylvaline (CEV) is the adduct formed by reaction of ACN with the N-terminal valine in human globin. This adduct is highly specific for exposure to ACN and, because it is built in erythrocytes, follows zero order kinetics, gradually disappearing as the erythrocyte pool is being replaced, i.e. after 126 days in humans (Granath et al., 1992). Other biomarkers of exposure exist for ACN but they have shorter half-lives or are less specific (Schettgen et al., 2012; Wu et al., 2012). Hence, the measurement of CEV in blood allows to carry out a biomonitoring program specifically for ACN over a more extended period of time. Consequently, CEV has been recommended as the biomarker of choice for chronic as well as for acute ACN exposure (Osterman-Golkar et al., 1994; Van Sittert et al., 1997; Bader and Wrbitzky, 2006).

A biomonitoring study was set up 2–3 weeks after the train accident to assess the exposure to ACN in the residents and in the persons that assisted occupationally in the accident. The aims of this specific study are (1) to determine exposure to ACN by means of CEV adducts in the blood of the residents of Wetteren with the highest suspected exposure, and (2) to assess the geographical distribution pattern of ACN exposure.

2. Materials and methods

2.1. Study population and study area

The evacuation zone (EZ) was defined by the Crisis Management Cell. The different zones are depicted in Fig. 1. Zone 1 corresponds to the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident. Zone 2 was evacuated later, i.e. in the days following the accident, and included the streets parallel with the sewage system and the streets downwind of the train accident.

Three groups of adult inhabitants of the EZ were invited to participate in the biomonitoring study. A first group consisted of residents of zone 1 (group 'EZ1'). A second group consisted of residents of zone 2 that were known to have presented at the emergency services of the surrounding hospitals (group 'EZ2 Emerg'). A third group consisted of a 10% sample of the residents of zone 2 that had been evacuated, but had not visited the emergency services (group 'EZ2 Evac'). This 10% sample was taken with the household as sampling unit: in a same household, the person who was the first to have his birthday following the accident was selected. In case the selected person was unable to attend the sampling, another member of the household was offered to participate in the biomonitoring program. Finally, residents of Wetteren living outside the predefined EZ, and who had visited the emergency services in the surrounding hospitals, were also eligible for the biomonitoring study (group 'Controls').

Table 1 presents the descriptive statistics of the study population. Blood, urine and questionnaires were collected from 242 (51.1%) of the eligible 474 residents. The participation rate varied between 47.5% and 65.0% among the subgroups of the EZ (groups 'EZ1', 'EZ2 Emerg' and 'EZ2 Evac'), but was lower in the residents living outside the EZ who had visited the emergency services (38.8% in the 'Controls'). Of the 242 participants, 41.3% were men, and the median age was 45.0 years. The median age was almost identical among the three subgroups of the EZ (respectively 48.5, 47.0 and 48.0 years in groups 'EZ1', 'EZ2 Emerg' and 'EZ2 Evac'), but was lower in the residents living outside the EZ who had visited the emergency services (34.0 years in the 'Controls').

2.2. Data collection

Blood, urine and questionnaires were collected from May 18–25, i.e. days 14 till 21 after the train accident with the assistance of the local general practitioners and the physicians of the Federal Public Service Health, Food Chain Safety and Environment. The study protocol was approved by the Ethical Committee of Ghent University Hospital and an informed consent was signed by all participants prior to their participation in the study.

Venous blood was sampled from each participant in 10 mL BD Vacutainer tubes containing EDTA (BD Vacutainer, ref. 367,525). Participants also provided a urine sample for the measurement of cotinine as biomarker for tobacco smoke exposure (Benowitz et al., 2009). It was measured to account for a person's smoking status because ACN is also present in tobacco smoke and smoking may thus interfere with the interpretation of the CEV measurements. Finally, each participant also filled in a short questionnaire. The questionnaire included (i) demographic variables, i.e. name, address, gender, day, month and year of birth; (ii) lifestyle variables, i.e. smoking status (non-smoker, ex-smoker, occasional smoker or daily smoker); and (iii) some specific variables related to the sampling, i.e. the day and the hour at which blood and urine sampling took place. After the results were available, an additional interview was taken from the group of the 'Controls' who showed CEV values above the reference values (see below). This interview allowed assessing (i) whether the study participants had been in the specific streets of the EZ at the time of and/or in the days following the train accident, and (ii) whether they were occupationally exposed to ACN in daily life.

2.3. Biomonitoring

Blood samples were pre-treated within 24 h to obtain a lysate of erythrocytes. The pretreated samples were stored at −20° C. Because of the need for substantial analysing capacity, blood samples were sent on dry ice to three different laboratories specialized in CEV analyses where a modified Edman degradation

Table 1
Eligible population, participation, age and gender distributions of the local study population ($n = 242$).

	EZ1	EZ2 Emerg	EZ2 Evac	Controls	Total
Eligible (*n*)	40	99	219	116	474
Participants(*n*, %)	26 (65.0)	47 (47.5)	124 (56.6)	45 (38.8)	242 (51.1)
Participants (*n*)	26	47	124	45	242
Age (years) Median (IQR)	48.5 (39.3–68.5)	47.0 (34.0–57.3)	48.0 (33.3–61.0)	34.0 (24.5–46.5)	45.0 (32.0–58.5)
Men *n* (%)	13 (50)	19 (40.4)	51 (41.1)	17 (37.8)	100 (41.3)

EZ1 Residents of zone 1 of the EZ, i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident.
EZ2 Residents of zone 2 of the EZ, i.e. the streets parallel with the sewage system and downwind of the train accident that was evacuated in the days following the accident.
EZ2 Emerg Residents of EZ2 that were known to have presented at the emergency services.
EZ2 Evac Residents of EZ2 that were evacuated, but did not visit the emergency services (10% sample).
Controls Residents of Wetteren, living outside the predefined EZ, that have visited the emergency services.
IQR: interquartile range.

Table 2
Non-extrapolated CEV concentrations (pmol/g globin) and *Q*-scores in the additional inter-laboratory comparison.

CEV concentration (pmol/g globin)			*Q*-scores		
Lab I	Lab II	Lab III	Lab I	Lab II	Lab III
NA	113.9	151.8	NA	−0.143	0.143
NA	517.1	519.5	NA	−0.002	0.002
NA	95.7	98.2	NA	−0.013	0.013
NA	71.7	76.7	NA	−0.034	0.034
NA	188.6	199.4	NA	−0.028	0.028
NA	168.9	209.0	NA	−0.106	0.106
NA	371.7	424.3	NA	−0.066	0.066
NA	740.2	811.3	NA	−0.046	0.046
NA	60.5	58.9	NA	0.013	−0.013
NA	129.9	150.9	NA	−0.075	0.075
232.0	283.0	NA	−0.099	0.099	NA
893.0	1028.0	NA	−0.070	0.070	NA
1062.0	1251.0	NA	−0.082	0.082	NA
357.0	427.0	NA	−0.089	0.089	NA
1505.0	1822.0	NA	−0.095	0.095	NA
46.0	64.0	NA	−0.164	0.164	NA
1124.0	1477.0	NA	−0.136	0.136	NA
44.0	58.0	NA	−0.137	0.137	NA
42.0	56.0	NA	−0.143	0.143	NA
418.0	549.0	NA	−0.135	0.135	NA
60.0	NA	84.5	−0.170	NA	0.170
60.0	NA	78.3	−0.132	NA	0.132
454.0	NA	608.0	−0.145	NA	0.145
168.0	NA	209.3	−0.109	NA	0.109
617.0	NA	709.2	−0.070	NA	0.070
116.0	NA	155.0	−0.144	NA	0.144
1272.0	NA	1499.2	−0.082	NA	0.082
11202.0	NA	14936.1	−0.143	NA	0.143
1155.0	NA	1481.2	−0.124	NA	0.124
4396.0	NA	5436.6	−0.106	NA	0.106

was used for adduct dosimetry (Van Sittert et al., 1997; Tornqvist et al., 1986). Blood samples taken between May 18 and 19 were sent to Lab I, between May 20 and 22 to Lab II, and between May 23 and 25 to Lab III. All three laboratories applied *N*-2-cyanoethyl-valine-leucine-anilide (Bachem, Bubendorf, Switzerland) for the calibration of the quantitative Edman procedure. Moreover all three laboratories participated successfully in the G-EQUAS inter-laboratory comparison before (Göen et al., 2012). The LLOQ's (lower limit of quantification) were respectively 0.5 (Lab I), 4.0 (Lab II) and 2.0 (Lab III) pmol/g globin. When receiving the results from the labs at the end of July, some CEV concentrations showed to be strongly increased (>1000 pmol/g globin, see further). To verify the results, we decided to carry out an extra inter-laboratory performance test at that moment. Therefore, 10 samples per laboratory were chosen, i.e. the 5 highest concentrations and 5 randomly lower concentrations. The 10 samples of the Lab I batch were sent to Lab II, the 10 samples of the Lab II batch were sent to Lab III, and finally, the 10 samples of the Lab III batch were sent to Lab I. Table 2 presents the CEV concentrations as measured on the sampling date and, for each pair of samples, the *Q*-scores (Hund et al., 2000). The *Q*-scores were calculated by the following formula:

$$Q\text{-score}_i = \frac{(\text{lab specific measure}_i - \text{mean of measure}_i)}{\text{mean of measure}_i}$$

Q-scores may be used as an alternative type of score in case *z*-scores cannot be calculated because the true value of the sample is unknown, as is the case in this additional inter-laboratory study. These *Q*-scores were then included in one-way ANOVAs with and without the factor 'laboratory'. The one-way ANOVA including the factor 'laboratory' showed a residual standard deviation of 6.5%. This is the best estimation of the mean standard deviation within a laboratory. The one-way ANOVA without the factor 'laboratory' showed a residual standard deviation of 11%. This is the best estimate for the total standard deviation due to inter-and intra-laboratory variance. As may be observed from Table 2, the additional inter-laboratory test revealed comparable results.

Smokers and non-smokers were identified based on cotinine in urine samples (De Cremer et al., 2013) and using a cut-off of 100 μg/L (Benowitz, 1996). Table 3 depicts the results. Seventy-four participants were categorized as 'smokers' and 168 were categorized as 'non-smokers'. This categorization was consistent with the reported (non-) smoking behaviour of the participants. While the proportion 'smokers' in the subgroups of the EZ ('EZ1', 'EZ2 Emerg' and 'EZ2 Evac') lay between 23.1 and 29.8%, it was 42.2% in the residents outside the EZ that had visited the emergency services (group 'Controls'). Consistent with this observation, the median urinary cotinine levels were markedly higher in smokers of the 'Controls' group (median: 1654 μg/L, IQR between 1224 and 2062 μg/L) when compared to smokers of the EZ (median: 1154 μg/L, IQR between 660 and 1439 μg/L) (data not shown).

2.4. Statistical analyses

CEV concentrations as measured in the blood were extrapolated back to the concentration that was to be expected at the time of the accident, i.e. May 4. Taking into account the average lifecycle of erythrocytes of 126 days, CEV values following a single exposure will decrease daily 1/126th (or 0.8%) until the background value (Granath et al., 1992; Bader and Wrbitzky, 2006). Based on these extrapolated CEV concentrations, the proportions above the reference value were used. For the non-smokers, the reference value is clearly defined in the literature, i.e. 10 pmol/g globin (Kraus et al., 2009). In contrast, for smokers, the reference value in the general population is less unequivocal (Kraus et al., 2009). For this study, an extrapolated CEV concentration of 200 pmol/g globin was used as cut-off for the smokers.

Table 3
Definition of the smoking status of the local study population ($n = 242$).

	Urinary cotinine (μg/L)	EZ1 ($n = 26$)	EZ2 Emerg ($n = 47$)	EZ2 Evac ($n = 124$)	Controls ($n = 45$)	Total ($n = 242$)
Smokers *n* (%)	>100	6 (23.1)	14 (29.8)	35 (28.2)	19 (42.2)	74 (30.6)
Non-smokers *n* (%)	≤100	20 (76.9)	33 (70.2)	89 (71.8)	26 (57.8)	168 (69.4)
	<25	20	32	87	25	164
	25–100	0	1[a]	2[b]	1[c]	4

EZ1 Residents of zone 1 of the EZ, i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident.
EZ2 Residents of zone 2 of the EZ, i.e. the streets parallel with the sewage system and downwind of the train accident that was evacuated in the days following the accident.
EZ2 Emerg Residents of EZ2 that were known to have presented at the emergency services.
EZ2 Evac Residents of EZ2 that were evacuated, but did not visit the emergency services (10% sample).
Controls Residents of Wetteren, living outside the predefined EZ, that have visited the emergency services.
[a] 1 self-reported 'ex-smoker'; [b]1 self-reported 'ex-smoker' and 1 self-reported 'non-smoker'; [c]1 self-reported 'non-smoker'.

Table 4
CEV concentrations, extrapolated at the moment of the train accident (pmol/g globin), in the non-smokers of the local study population ($n = 168$).

Non-smokers	EZ full ($n = 142$)	EZ1 ($n = 20$)	EZ2Emerg + Evac ($n = 122$)	EZ2 Emerg ($n = 33$)	EZ2 Evac ($n = 89$)	Controls ($n = 26$)	Controls corrected for localisation ($n = 24$)
Mean (SD)	206.7 (1163.4)	13.7 (15.4)	238.9 (1253.0)	662.8 (2325.0)	80.9 (317.0)	71.3 (337.4)	4.3 (3.3)
Median (IQR)	6.9 (3.4–16.8)	9.9 (4.0–14.4)	6.9 (3.4–18.1)	8.0 (5.7–67.6)	6.8 (3.4–15.8)	4.0 (2.3–6.6)	3.0 (2.3–5.7)
P95	634.6	35.9	1151.7	2760.6	339.5	21.9	8.2
Maximum	12614.8	64.8	12614.8	12614.8	2128.5	1725.5	16.2
>Ref. value n (%)[a]	53 (37.3)*	10 (50.0)**	43 (35.2)**	13 (39.3)***	30 (33.7)***	3 (11.5)	1 (4.2)*

EZ full Residents of the evacuation zone as determined by the Crisis Management Cell.
EZ1 Residents of zone 1 of the EZ, i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident.
EZ2 Residents of zone 2 of the EZ, i.e. the streets parallel with the sewage system and downwind of the train accident that was evacuated in the days following the accident.
EZ2 Emerg Residents of EZ2 that were known to have presented at the emergency services.
EZ2 Evac Residents of EZ2 that were evacuated, but did not visit the emergency services (10% sample).
Controls Residents of Wetteren, living outside the predefined EZ, that have visited the emergency services.
Controls corrected for localisation Results as obtained by the additional interview, correcting for misclassification by residential address and/or by occupational exposure to ACN.
SD: standard deviation; IQR: interquartile range.
*P value = 0.003; **P value = 0.310; ***P value = 0.711 (Chi-square tests).
[a] 10 pmol/g globin.

3. Results

3.1. Summary statistics of CEV concentrations in the local population

3.1.1. CEV concentrations in the EZ versus outside the EZ

CEV concentrations above the reference value of 10 pmol/g globin were observed in 37.3% of the non-smokers in the EZ as compared to 11.5% of the 'Controls' outside the EZ. The 95th percentiles were 635 and 22 pmol/g globin, respectively (Table 4). In contrast, in the smokers (Table 5), the proportion exceeding the reference value of 200 pmol/g globin was higher in the 'Controls' outside the EZ (68.4%) as compared to the EZ (40.0%). The 95th percentiles and the maxima, however, were higher in the EZ than in the 'Controls'.

To verify whether the CEV concentrations above the reference values in the group of 'Controls' outside the EZ could be explained by misclassification by residential address and/or by occupational exposure to ACN, an additional interview was done in all three non-smokers and in 8 of the 13 smokers. Two of the non-smokers and 5 of the 8 smokers reported they had been in the EZ at the time of or in the days following the train accident. None of the persons was working in the production of polymers, ... occupationally. Five smokers did not participate in the additional interview and thus kept the classification 'outside the EZ' as based on their residential address. After correction for localisation as obtained by the additional interview (Table 4), one (4.2%, CEV concentration of 16 pmol/g globin) of the remaining non-smokers outside the EZ had CEV concentrations above the reference value in contrast with 37.3% in the EZ (Chi-square test, P value = 0.003). In the remaining smokers outside the EZ (Table 5), 57.1% kept CEV concentrations above the reference value, as compared to 40.0% in the smokers of the EZ (Chi-square test, P value = 0.394).

3.1.2. CEV concentrations in the different zones of the EZ

In zone 1 (EZ1) and zone 2 (EZ2), 50.0% and 35.0% of the non-smokers (Table 4) had CEV values above the reference level of 10 pmol/g globin, respectively (Chi-square test, P value = 0.310). In zone 1 (EZ1), the concentrations did not exceed a remarkably low maximum of 65 pmol/g globin, the 95th percentile being 36 pmol/g globin. In contrast, in zone 2 (EZ2), the highest CEV concentrations of the whole local population were observed. This was the case for both the residents who were known to have presented at the emergency services ('EZ2 Emerg') and the 10% sample of residents who were evacuated and did not present at the

Table 5
CEV concentrations, extrapolated at the moment of the train accident (pmol/g globin), in the smokers of the local study population ($n = 74$).

Smokers	EZ full($n = 55$)	EZ1 ($n = 6$)	EZ2Emerg + Evac ($n = 49$)	EZ2 Emerg ($n = 14$)	EZ2 Evac ($n = 35$)	Controls ($n = 19$)	Controls corrected for localisation ($n = 14$)
Mean (SD)	212.1 (122.0)	230.3 (76.5)	209.9 (126.8)	200.6 (103.8)	213.6 (136.1)	223.2 (73.7)	202.2 (58.0)
Median (IQR)	183.0(149.5–272.5)	189.5(/)	181.3(131.8–265.2)	185.8(105.3–287.5)	174.3(141.6–264.6)	211.4(183.5–253.9)	203.0(160.9–237.5)
P95	402.4	/	431.0	355.6	477.3	329.3	292.0
Maximum	694.8	337.5	694.8	373.9	694.8	433.0	317.8
>Ref. value n (%)[a]	22 (40.0)*	2 (33.3)**	20 (40.8)**	6 (42.8)***	14 (40.0)***	13 (68.4)	8 (57.1)*

EZ full Residents of the evacuation zone as determined by the Crisis Management Cell.
EZ1 Residents of zone 1 of the EZ, i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident.
EZ2 Residents of zone 2 of the EZ, i.e. the streets parallel with the sewage system and downwind of the train accident that was evacuated in the days following the accident.
EZ2 Emerg Residents of EZ2 that were known to have presented at the emergency services.
EZ2 Evac Residents of EZ2 that were evacuated, but did not visit the emergency services (10% sample).
Controls Residents of Wetteren, living outside the predefined EZ, that have visited the emergency services.
Controls corrected for localisation Results as obtained by the additional interview, correcting for misclassification by residential address and/or by occupational exposure to ACN.
SD: standard deviation; IQR: interquartile range.
*P value = 0.394 (Chi-square test); **P value = 1.000 (Fisher-Exact test); ***P value = 1.000 (Fisher-Exact test).
[a] 200 pmol/g globin.

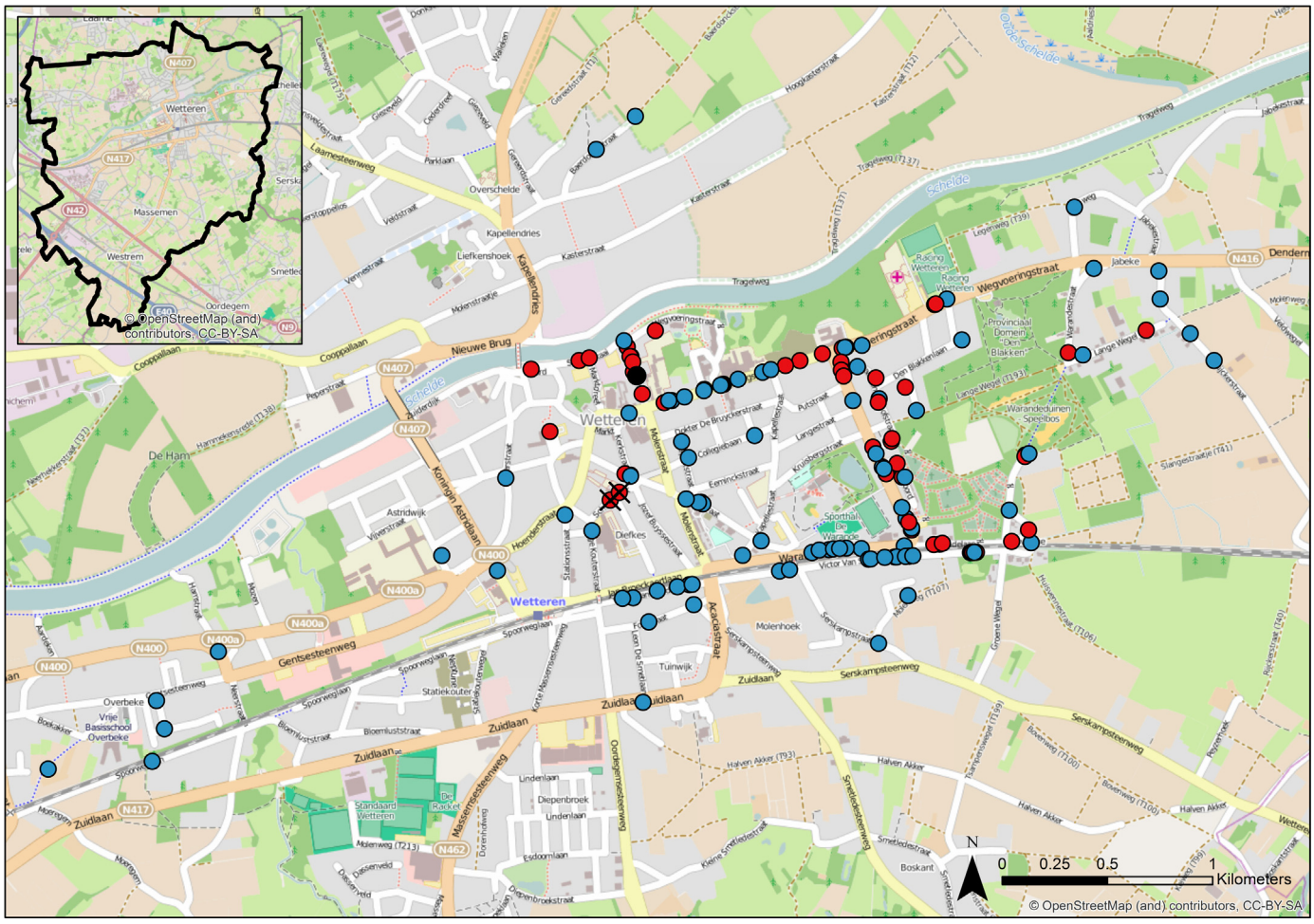

Fig. 2. Spatial distribution of the CEV concentrations extrapolated at the moment of the train accident (pmol/g globin) in the non-smokers of the local study population ($n = 168$).
Extrapolated CEV concentration ≤10 pmol/g globin.
Extrapolated CEV concentration > 10 pmol/g globin.
Has been in the EZ at the moment of or in the days following the train accident.
Extrapolated CEV concentrations of 4951 and 12615 pmol/g globin.

emergency services ('EZ2 Evac'), but was even more pronounced in the group 'EZ2 Emerg' (95th percentile and maximum of 2761 and 12 615 pmol/g globin, respectively) than in the group 'EZ2 Evac' (95th percentile and maximum of 340 and 2129 pmol/g globin, respectively).

In the smokers (Table 5), the proportions above the reference value of 200 pmol/g globin were similar between zone 1 ('EZ1′) and zone 2 ('EZ2′), as well as between the two groups of zone 2 ('EZ2 Emerg' and 'EZ2 Evac'). The maximum CEV concentration was 695 pmol/g globin and observed in the group of 'EZ2 Evac'.

3.2. Spatial distribution of CEV concentrations in the local population

Fig. 2 presents a spatial mapping, according to the residential address, of the 168 non-smokers. The results of the smokers were omitted because it was not possible to distinguish the CEV contribution by the accident from that resulting from smoking, because smokers already had a higher starting level. The CEV concentrations above the reference level in the non-smokers were largely concentrated in certain streets of the EZ. Apart from the street lining the railway; the other streets largely coincide with the route of the sewage system, demonstrating the highly peculiar, moving nature of this accident. The two extreme outliers in the non-smoking group (4951 and 12 615 pmol CEV/g globin), indicated on the map, were observed at the same address. As mentioned above (3.1.1.), CEV concentrations above the reference value were also observed in three non-smokers with residential address outside the EZ. When taking into account the information as obtained by the additional interview, the more extreme increases (1726 and 24 pmol/g globin) could be explained by the presence in the EZ at the moment of or in the days following the train accident. Only for one non-smoker with a CEV concentration of 16 pmol/g globin, it was not clear where the slightly increased level came from.

4. Discussion and conclusion

This study describes the results of the largest human biomonitoring study in the general population performed to date in order to assess accidental ACN exposure. The basis of exposure in this case was a train derailment at Wetteren, Belgium, which resulted in a highly atypical sequence-of-events. More specifically,

apart from possible exposure in the direct vicinity of the site of the train derailment, exposure was also possible via the sewage system, into which acrylonitrile had entered shortly after the accident. Concentrations of CEV, an adduct of ACN with the N-terminal valine of Hb, were measured in the blood of residents, amongst which those with the highest suspected exposure. Biological monitoring was carried out on residents of the evacuation zone (EZ), as determined by the Crisis Management Team, as well as on the residents living outside the EZ who had visited the emergency services. The EZ was subdivided in three subgroups, which were comparable with regard to age and smoking status. The residents living outside the EZ who had visited the emergency services, however, were younger, reported substantially more often smoking and were heavier smokers than the smokers of the EZ. The overall participation rate amounted to 51% which is acceptable for this type of study. The participation rate was comparable among the three subgroups of the EZ, but was lower in the residents outside the EZ. In non-smokers, 37.3% of the residents in the EZ showed CEV values above the pre-defined reference value of 10 pmol CEV/g globin, whereas in smokers the reference value of 200 pmol CEV g/globin was exceeded in 40.0%.

In the non-smokers, some clear patterns with regard to ACN exposure following the train accident were seen in function of the subgroups.

First, the evacuation zone (EZ) seems to have been determined well by the Crisis Management Team. Outside the EZ, CEV concentrations above the reference level were only observed in 4.2% of the non-smokers, which is in line with what is to be expected on the basis of the definition of the reference value, i.e. the 95th percentile in a non-exposed population.

Second, the timing of evacuation seems to have had an effect on the CEV concentrations, especially on the occurrence of higher concentrations. In zone 1 (EZ1), i.e. the 250 m perimeter of the EZ that was evacuated at night in the hours immediately following the accident, 50.0% of the non-smokers exceeded the reference level, but the CEV levels did not exceed a remarkably low maximum of 65 pmol/g globin. In Zone 2 (EZ2), i.e. the streets parallel with the sewage system and the streets downwind of the train accident that were evacuated in the days following the accident, 35.0% had values above the reference level. However, CEV concentrations with maxima in the order of magnitude of several thousands of pmol/g globin were observed. Whether these higher values reflect a more intense exposure or rather a more prolonged exposure (leading to accumulation of the biomonitoring parameter) is not known.

Third, the CEV concentrations above the reference value were observed in the street along the railway and particularly in the streets corresponding to the sewage system. Also the person who died following the accident, as well as the two persons presenting with life-threatening symptoms, lived in the streets along the sewage system. Consequently, the most important route of exposure to ACN for the residents seems to have been by inhalation of ACN vapours, either directly (immediate vicinity of the accident), or indirectly via the sewage system. Differences in CEV concentrations were observed between the residents of zone 2 who had presented at the emergency services ('EZ2 Emerg') and the 10% sample of residents of zone 2 who had been evacuated, but did not present at the emergency services ('EZ2 Evac'). Both groups are living in the same streets along the sewage system and were thus evacuated during the same period of time. In the group 'EZ2 Emerg', maxima of 4951 and 12 615 pmol/g globin were observed, whereas in the group 'EZ2 Evac' the maximum was 2129 pmol/g globin. The differences in the two populations along the sewage system may be due to (i) differences in connection to the sewage system and/or (ii) different places of dwelling in the building of the individuals.

In the smokers, the proportion of individuals with CEV concentrations above 200 pmol CEV/g globin was higher outside the EZ (57.1%) than in the EZ (40.0%), even after (partial) correction for localisation. As cotinine determinations revealed that the former were heavier smokers, it is likely that the difference in tobacco smoke exposure underlies this observation. The apparent high proportion of smokers considered as "positive" in this study can be linked to the defensively chosen cut-off of 200 pmol CEV/g globin. Indeed, one may argue that this cut-off is too low, given the fact that variation exists, with ranges described between 146 pmol/g globin and 332 pmol/g globin, mainly determined by the extent of tobacco consumption (Kraus et al., 2009). In contrast to the non-smokers of the EZ, no clear pattern could be distinguished among the different subgroups of the smokers in the EZ. Ideally, for every individual smoker, a personal background value should be known to draw conclusions and still then, it is likely that the CEV background imposed by tobacco exposure will mask a mild exposure to ACN. Hence, no formal conclusions can be inferred from the CEV values observed in smokers.

Biological monitoring following chemical disasters has been recommended as part of disaster management in order to objectivate the internal human exposure (Scheepers et al., 2011). To the authors' knowledge, two previous studies have reported on biological monitoring of CEV following accidental ACN exposure. The CEV values reported in these studies were substantially lower than the CEV concentrations measured in the current study. Following the death of a cleaning worker after decontamination of an ACN containing tank wagon, Bader and colleagues (Bader and Wrbitzky, 2006) reported CEV concentrations of 679 pmol/g globin (non-smoker) and 768–2424 pmol/g globin (smokers) in the co-workers. In the rescue workers and medical staff who tried to resuscitate the person, no increased CEV concentrations were observed. In another German study (Leng, 2009), CEV monitoring was carried out on 600 persons from fire brigades, police and rescue organisations after a fire in an ACN tank of a chemical plant in 2008. In 99% of the sampled population, body burden was $<$40.8 pmol/g globin for non-smokers and $<$612 pmol/g globin for smokers.

In this study, exposure to ACN was assessed by measuring CEV in the blood as this adduct is generally accepted as the best choice biomarker for ACN exposure. CEV was thus used as a tool to reconstruct the exposure at the moment of the train accident. Indeed, the lifetime of the erythrocytes in the human body is long (126 days) and as there are no repair mechanisms for haemoglobin adducts, their quantification offers the unique possibility to explore even past high exposures or chronic low level exposures (Schettgen et al., 2010). The results found in this study are in excellent accordance with duration and assumed intensity of exposure, respectively. Thus, the recent study confirms the applicability of the biomonitoring approach for risk assessment and studying the causality of effects of the victims of such a chemical disaster.

Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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